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TARGETING LYSOPHOSPHOLIPID S1P RECEPTORS FOR THE TREATMENT OF MULTIPLE SCLEROSIS

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SUMMARY

Sphingosine 1-phosphate (S1P) is an example of a lysophospholipid, a class of bioactive lipids with extracellular effects mediated by G protein-coupled receptors. There are five known S1P receptor subtypes, $S1P_{1\text{-}5}$. S1P receptors have gained increasing relevance for the therapeutic treatment of multiple sclerosis (MS) through studies of a breakthrough compound, FTY720, (known clinically as fingolimod), which has the potential to become the first oral therapy for the first-line treatment of relapsing–remitting MS. FTY720 is a prodrug that, when phosphorylated in vivo, becomes a nonselective S1P receptor agonist at four of the five receptors, $S1P_{1,3,4,5}$, supporting receptor modulation at one or more of these S1P receptors in treating MS, although receptor subtypes and forms of modulation may be varied and diverse. Emerging understanding of the receptor mechanism of action in MS suggests possible strategies for going forward, although this is complicated by uncertainties over the complete mechanism of action that may interrupt not only inflammatory insults, but may also directly modulate central nervous system activities. Nevertheless, robust experimental and clinical experience with FTY720 portends the development of new compounds having improved efficacy and/or safety profiles, which could herald a new generation of oral MS therapies based on S1P receptor modulation.

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INTRODUCTION

Multiple sclerosis (MS) is the most common cause of demyelinating pathology and neurological impairment in young adults, afflicting an estimated 2.5 million individuals throughout the world (1, 2). MS is considered to be a chronic autoimmune disorder that affects the central nervous system (CNS), resulting most commonly in a relapsing–remitting presentation associated with a broad range of neurological and psychiatric symptoms, demyelination and, perhaps most significantly, neurodegenerative changes that can lead ultimately to premature death. The causes of MS remain unclear, with a relatively weak genetic linkage, but with the most common forms showing epidemiological bias for women, particularly those living in North America and Europe (1-4).

There are four major clinical presentations of MS that have been generally recognized (1, 2, 4, 5): the most common is relapsing–remitting MS (RRMS); this can progress to secondary progressive MS (SPMS); a rare distinct form, primary progressive MS (PPMS), in which debilitation is not associated with remission; and a progressive–relapsing MS (PRMS), where progressive debilitation is accentuated by exacerbation followed by some degree of recovery. The etiological and mechanistic relationships among these forms of MS remain unclear, aside from the accepted involvement of at least some degree of immunological insult that is associated with CNS damage, particularly demyelination and axonal loss (4-8). The predominant view is of MS as a primarily immune-driven process that produces inflammatory demyelination and, over time, neurodegenerative changes. Damage is thought to commence when autoimmune lymphocytes penetrate the blood–brain barrier (BBB), resulting in damage within the CNS parenchyma. However, there exists an alternative view that one or more of the four subforms of MS may primarily be a neurodegenerative disease that is accompanied secondarily by immunological responses (3, 4, 9). The distinction between these mechanisms has therapeutic implications for future drug development, particularly in view of the current state of MS therapeutics that target immune-mediated inflammatory aspects of MS to reduce relapses, but which have unclear long-term benefits in preventing CNS damage, along with resulting neurological and psychiatric impairment (3-5).

CURRENT DISEASE-MODIFYING THERAPIES

First-line disease-modifying therapies (DMTs) to treat MS that are in current use all target the immune system. Primary examples are the

interferons (IFNs) (10-12): IFN-β-1a (Avonex®, Rebif®) and IFN-β-1b (Betaseron®, Extavia®), which in the U.S. are FDA-approved for RRMS. A distinct DMT is glatiramer acetate (Copaxone®), an amino acid polymer that also has an unclear mechanism of action but appears to target the immune system (13, 14). Second-line DMTs also target the immune system. The humanized monoclonal antibody natalizumab (Tysabri®) (15) is directed against the cell adhesion molecule α_4 integrin that is normally involved in leukocyte adhesion and migration (16). This DMT, delivered by i.v. infusion, shows improved efficacy over other therapies in RRMS in reducing relapses, although it has been clearly associated with a potentially lethal brain infection, progressive multifocal leukoencephalopathy (PML) (17, 18). A distinct, second-line DMT is mitoxantrone (Novantrone®) (14, 19), a cytotoxic, antimitotic chemotherapeutic compound that also appears to have immunomodulatory effects. It too requires delivery by i.v. infusion and is FDA-approved for SPMS, PRMS, as well as worsening RRMS, but shows dose-limiting cardiac toxicity and a risk for acute myeloid leukemia (AML), as well as other side effects (19, 20). There are no FDA-approved therapies for PPMS.

All of these therapies are administered via injection or i.v. infusion. These agents are also, as a whole, thought to target immunological components of MS towards disrupting CNS inflammation. In addition, all first-line therapeutics are not small molecules, instead being peptidergic (interferons, glatiramer acetate or the antibody natalizumab), and none are utilized as oral formulations.

THE LYSOPHOSPHOLIPID SPHINGOSINE 1-PHOSPHATE (S1P) AND ITS RECEPTORS

A fundamentally different approach to treating MS may be through targeting receptors for S1P. S1P is a member of a class of membrane-derived signaling lipids known as lysophospholipids (which prominently include S1P and lysophosphatidic acid [LPA]) that are characterized by a 3-carbon backbone, a single linked aliphatic carbon chain of varied length and saturation, and a phosphate head group (21, 22). S1P is produced by intracellular phosphorylation of sphingosine by sphingosine kinases (Fig. 1) (23).

S1P has reported intracellular activities (24), but its best validated biological and pathophysiological effects are mediated by binding of extracellular S1P, after transport out of the cell, to cognate G protein-coupled receptors (GPCRs) (22, 25-27). There are five proven S1P receptors, named $S1P_{1-5}$ (Fig. 2) (21, 22, 28). Upon ligand binding, these receptors activate heterotrimeric G proteins, which in turn activate multiple downstream signaling pathways.

An extensive range of cellular activities have been identified as receptor-mediated, including survival, proliferation, morphological, electrophysiological and migratory, among others, which in turn affect organism development in both normal and pathophysiological processes (22, 25-29). Many insights have emerged from in vivo studies through the creation and analysis of knockout mice for four of the five S1P receptors (22, 30-33).

S1P RECEPTORS AS THERAPEUTIC TARGETS IN MULTIPLE SCLEROSIS: FTY720

S1P receptors and most other lysophospholipid GPCRs are widely expressed on cells that have been implicated in the etiology and progression of MS. These include many immunological cell types, such as lymphocytes (29, 34-36), nervous system cells that include neurons (30, 34, 37-40), astrocytes (41-43), myelinating cells (44-47) and resident microglia (48, 49), as well as vascular cell types (37, 46, 50, 51). Most importantly, the entry of FTY720 (fingolimod) into the field of lysophospholipid signaling has raised the real possibility that S1P receptor modulation could indeed be therapeutically useful. This compound was chemically modified from a fungal natural product, myriocin (Kyoto University, Yoshitomi) (25, 52), and licensed to Novartis as an orally bioavailable agent for potentially preventing organ transplant rejection, based on animal studies supporting immunosuppressive activities through an unclear mechanism of action (53). Additional studies demonstrated its relevance to S1P receptor signaling through the identification of the active phosphorylated metabolite, FTY720-P, which was shown to be a nonselective, high-affinity S1P receptor agonist at each of the S1P receptors except $S1P_2$ (54, 55). This metabolite is produced by the in vivo action of sphingosine kinases, particularly sphingosine kinase 2, on FTY720 (56) (Fig. 3).

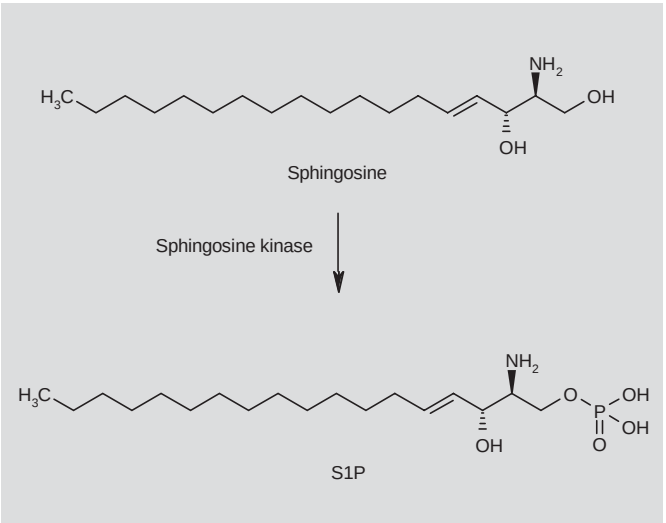

Figure 1. Chemical structures of sphingosine and sphingosine 1-phosphate (S1P), which is produced by phosphorylation of sphingosine by sphingosine kinases (sphingosine kinase 1 or 2).

Immunosuppressive activities also supported the possible use of this compound in MS, and animal models demonstrated efficacy consistent with this view (55, 57, 58). Human clinical trials for kidney transplant progressed through phase III but did not show benefit over standard-of-care treatments (59). However, distinct clinical trials to assess oral FTY720 (referred to in MS clinical trials as fingolimod) efficacy in RRMS indeed showed efficacy, and this small-molecule compound has now progressed through phase II and multiple phase III trials (60-63). As of this writing, it has received FDA fast track designation and Advisory Committee unanimous recommendation as a new molecular entity (NME) that could represent the first oral disease-modifying treatment for RRMS.

The mechanism of action of FTY720/fingolimod is thought to involve immunological modulation by the active metabo-

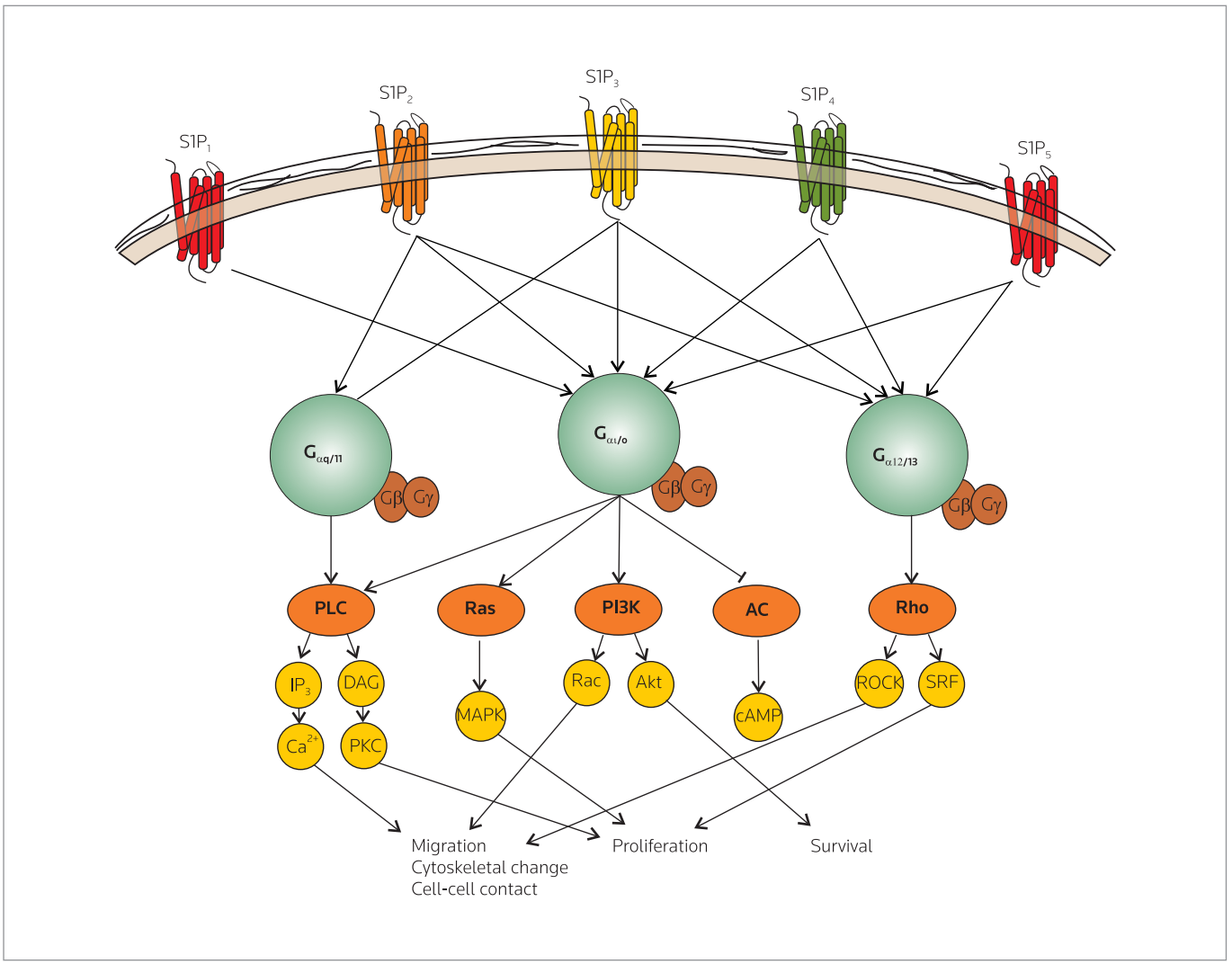

Figure 2. S1P receptors and their downstream signaling pathways. Each of the five S1P receptors is a G protein-coupled receptor (GPCR). Following ligand binding, a receptor interacts with heterotrimeric G proteins that activate a range of downstream signaling pathways. Major G protein pathways are illustrated; in addition, interactions with $G_{\alpha s}$ have been reported for some receptors (not shown).

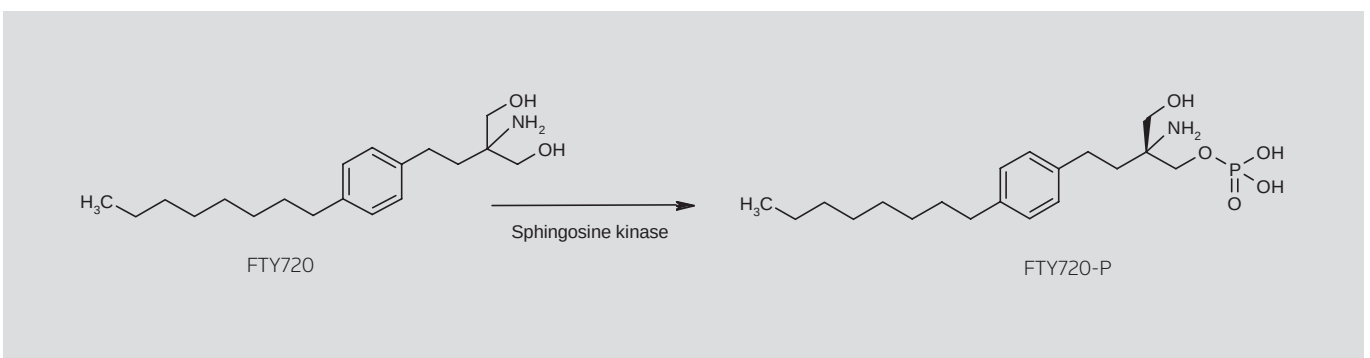

Figure 3. Chemical structures of FTY720, a prodrug, which is phosphorylated to produce the active compound, FTY720-P. Phosphorylation occurs predominantly via sphingosine kinase 2, although sphingosine kinase 1 can also produce some degree of phosphorylation.

lite of one or more of the activated S1P receptors, particularly the $S1P_1$ subtype. Genetic studies using knockout mice have shown the importance of $S1P_1$ for normal lymphocyte trafficking (29, 64, 65). These studies further indicated that the likely mechanism of action involved "functional antagonism", whereby FTY720-P binding produces initial agonism followed by irreversible $S1P_1$ internalization. In this setting, unlike typical agonist binding produced by S1P itself, which results in receptor recycling back to the cell surface, internalization by FTY720-P produces ubiquitin-mediated receptor degradation (66), effectively removing $S1P_1$ and phenocopying a receptor-null knockout. This hypothesis is complicated by data supporting the activity of $S1P_1$ even after it is internalized but preceding its degradation (67).

These aspects of receptor modulation are relevant in considering the nature of how an agent influences a given S1P receptor, which could formally include one or more activities as an agonist, antagonist, functional antagonist, or possibly as an inverse agonist, based upon FTY720 effects. An additional variable to consider is the tissue distribution of FTY720, which appears to localize preferentially within the CNS (68). This is likely to be relevant to a range of in vitro studies that support possible CNS effects, including nervous system expression and responses of S1P receptors in tissue sections, as well as activities of receptor modulators in cell culture (69, 70). This possibility receives correlative in vivo support through a discordance between disease severity that did not track with FTY720-mediated immunological endpoints observed in animal models of MS (57), as well as an inverse relationship between FTY720/fingolimod dose and observed efficacy in MS, whereby the lowest dose showed comparable or increased efficacy, as well as improved safety, compared to the highest drug dose (60, 61, 71). Functional assessment of a putative nervous system mechanism of action awaits further studies.

FUTURE S1P RECEPTOR TARGETS

Based on experimental and clinical experience with FTY720, four of the five S1P receptors could be targets for new MS therapies: $S1P_{1,3,4,5}$ (72). Formally, $S1P_2$ has not been validated in the literature for activities relevant to MS therapeutics, and is being included here for completeness. Some additional aspects of these receptors are noted below. The human and murine gene names, respectively, are noted in parentheses. All are 7-transmembrane domain GPCRs. Of paramount importance in considering these receptors as mono- or polyreceptor-based targets is the safety of an assessed molecular entity, which could produce both mechanism-based and non-mechanism-based effects and/or toxicities. The unusual properties of FTY720 as a prodrug that localizes in the CNS to produce a nonselective S1P receptor agonist that can also act as a functional antagonist leave open the question of how to improve upon oral FTY720/fingolimod, representing both a challenge as well as an opportunity for future drug design in the therapeutic treatment of MS, or perhaps other S1P receptor-influenced disorders.

$S1P_1$ *(S1PR1, S1pr1)*

$S1P_1$ has the greatest degree of validation as a target for influencing the course of MS, in view of data from both FTY720-P studies, as well as genetic approaches. It represents the first functionally identified S1P receptor (50; reviewed in 21, 22). Located on chromosome 1, it is encoded by a single exon to produce a receptor of 381 amino acids. Upon ligand binding, $S1P_1$ normally appears to couple exclusively to the heterotrimeric G protein $G_{\alpha i/o}$. Its relevance to MS includes demonstrated roles in the trafficking of lymphocytes (64), as well as other hematopoietic cells (25, 27, 29). In addition, its normal function contributes to maintaining vascular barrier integrity (27). The expression of $S1P_1$ in neural cells may contribute to the efficacy of FTY720. Follow-up compounds such as BAF312 (Novartis) (73) and distinct compounds from multiple academic and commercial entities (e.g., the $S1P_1$-selective agonist ACT-128800 from Actelion, being evaluated for RRMS) should provide further testing of the importance of this receptor subtype in MS therapeutics. It will be of particular interest to examine in detail neural as compared to inflammatory endpoints (3) in assessing overall efficacy of these agents in MS.

$S1P_2$ *(S1PR2, S1pr2)*

This receptor has the least validation with respect to MS since it is the one S1P receptor subtype that is not effectively modulated by FTY720-P. It has not been formally tested in human MS, and therefore remains of uncertain therapeutic relevance. $S1P_2$ is encoded by a single-exon gene located on chromosome 19, translated into a 353-amino-acid GPCR that couples to $G_{\alpha i/o}$, $G_{\alpha q}$ and $G_{\alpha 12/13}$, with a few reports of $G_{\alpha s}$ coupling as well (22, 25, 27). Functions in vascular integrity (74, 76), as well as neural activities (22, 47, 77, 78), leave open the possibility that activities relevant to MS may be identified. This possibility receives further support through reported genetic interactions between $S1P_2$ and $S1P_3$ in the maintenance of neural-mediated hearing and balance (78).

$S1P_3$ *(S1PR3, S1pr3)*

Located on chromosome 9 and encoded on a single exon, this 378-amino-acid receptor couples to the heterotrimeric G proteins $G_{\alpha i/o}$, $G_{\alpha q}$ and $G_{\alpha 12/13}$, and, like $S1P_2$, a few reports suggest $G_{\alpha s}$ coupling (22, 25, 27). Its relevance to MS relates primarily to the promiscuous S1P receptor activities of FTY720-P, although there have been no reported validation studies in humans using monospecific $S1P_3$-selective compounds. An interesting relationship exists between $S1P_3$ and Sandhoff disease –a lysosomal lipid storage disorder producing neurodegeneration– whereby pathology in a mouse model can be attenuated by removal of $S1P_3$ signaling (79). A prominent component of both Sandhoff disease and MS is astrogliosis (79-82), raising the possibility that $S1P_3$ receptor inactivation could help to reduce astrogliotic changes in MS.

$S1P_4$ *(S1PR4, S1pr4)*

The gene for the fourth S1P receptor resides on chromosome 19, as do those for $S1P_2$ and $S1P_5$. The 384-amino-acid protein couples to $G_{\alpha i/o}$, $G_{\alpha 12/13}$, and possibly $G_{\alpha s}$ (22, 25, 27). Expression of $S1P_4$ has been reported on lymphoid cells (35), where receptor activation can potentially produce immunosuppressive effects (83). Aside from agonism by FTY720-P, validation for modulation of the receptor remains unassessed.

$S1P_5$ *(S1PR5, S1pr5)*

The fifth S1P receptor is also encoded by a single-exon gene located on chromosome 19. It couples to the heterotrimeric G proteins $G_{\alpha i/o}$

and $G_{\alpha 12/13}$, and possibly others (22, 25, 27). Its relevance to MS includes expression on natural killer cells, the trafficking of which is disrupted by receptor deletion (32, 33), which further involves lymphoid T-bet/TBX21 transcription factor mechanisms. In addition, gene expression for this receptor has been reported on oligodendroglia (84, 85), raising the possibility that some aspects of FTY720 efficacy may involve actions at receptors for both immunological and CNS cells. Some human proof-of-concept studies may be under way based on the compound BAF312, which could be a modulator of both $S1P_1$ and $S1P_5$. This suggests a closer functional linkage revealed by biology and chemistry between $S1P_5$ and $S1P_1$, making it a receptor of particular interest that awaits further assessment.

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